

E.coli HB101 (pTSBH) and HB101 (pTSBG1) have been deposited with the National Institute of Advanced Industrial Science and Technology International Patent Organism Depository (Address: Central 6, 1-1 Higashi 1-chome, Tsukuba City, 5 Ibaraki Prefecture, Japan) under the accession number FERM BP-7118 (deposition date: April 11, 2000) and the accession number FERM BP-7119 (deposition date: April 11, 2000), respectively.

Further, the plasmid pGDA2 (J. Biol. Chem., (1989), 10 264, 6381) was double-digested with EcoRI and PstI and the thus-obtained DNA fragment (about 0.9 kb) containing the Bacillus megaterium IWG3-derived GDH gene was inserted into the plasmid pSTV28 (product of Takara Shuzo) at the EcoRI-PstI site thereof to construct a recombinant plasmid pSTVG. 15 E.coli HB101 (pTSBH) made competent in advance by the calcium chloride method was transformed with this plasmid pSTVG at a high rate of introduction. Thus, E.coli HB101 (pTSBH, pSTVG) was obtained with ease.

20 (Example 9: BRD expression in recombinant E.coli)

The recombinants E.coli HB101 (pTBH) and HB101 (pTSBH) obtained in Example 8 were each shake-cultured on 2 X YT medium containing 200 µg/ml of ampicillin at 28°C for 15 hours. A 1-ml portion of this preculture fluid was 25 inoculated into 100 ml of a medium sterilized by autoclaving in a 500-ml Sakaguchi flaks and comprising 1.5% (w/v) glycerol, 1.5% (w/v) Bacto tryptone, 0.4% (w/v) Bacto yeast extract, 0.2% (w/v) sodium chloride, 0.8% (w/v) potassium dihydrogen phosphate, 0.05% (w/v) magnesium sulfate heptahydrate, and 0.033% (w/v) Adekanol LG109 (product of Asahi Denka Kogyo), as adjusted to pH 6.0, and shake culture was carried out at 30°C for 60 hours. Cells 30 were harvested from such culture fluids by using a

RESULT 4

E64938

probable aldehyde reductase (EC 1.1.1.-) b1781 - Escherichia coli (strain K-12)

C;Species: Escherichia coli

C;Date: 10-Sep-1999 #sequence\_revision 10-Sep-1999 #text\_change 01-Mar-2002

C;Accession: E64938

R;Blattner, F.R.; Plunkett III, G.; Bloch, C.A.; Perna, N.T.; Burland, V.; Riley, M.; Collado-Vides, J.; Glasner, J.D.; Rode, C.K.; Mayhew, G.F.; Gregor, J.; Davis, N.W.; Kirkpatrick, H.A.; Goeden, M.A.; Rose, D.J.; Mau, B.; Shao, Y. Science 277, 1453-1462, 1997

A;Title: The complete genome sequence of Escherichia coli K-12.

A;Reference number: A64720; MUID:97426617; PMID:9278503

A;Accession: E64938

A;Status: nucleic acid sequence not shown; translation not shown

A;Molecule type: DNA

A;Residues: 1-284 <BLAT>

A;Cross-references: GB:AE000273; GB:U00096; NID:g1788078; PIDN: AAC74851.1; PID:g1788081; UWGP:b1781

A;Experimental source: strain K-12, substrain MG1655

C;Superfamily: aldehyde reductase

C;Keywords: oxidoreductase

Query Match 51.7%; Score 752; DB 1; Length 284;  
Best Local Similarity 52.1%; Pred. No. 3.5e-57;  
Matches 147; Conservative 48; Mismatches 81; Indels 6; Gaps 3;

Qy 2 RRMTLPSGE-SIPVLGQGTWGWGEDPGRRGDEVAALHAGLELGMTLVDTAEMYADGGAEE 60  
::| ||: |::| :||||| |||| :| ||||| ||:|||:||:|||||||:|||:  
Db 3 QKMIQFSGDVSLPAVGQGTWYMGEDASQRKTEVAALRAGIELGLTLIDTAEMYADGGAEK 62

Qy 61 VAGEALAGRDEAFVVSKVMPSHASRSGTIAACERSLKR LGTDRIDL YLLHWQGRYPLQD 120  
| ||||| | |::| :||||| | :| | ||||| ||:||| | :| :||| | | : ::  
Db 63 VVGEALTGLREKVFLVKVYPWNAGGQKAINACEASLRLN TDYLDLYLLHWSGSF AFE 122

Qy 121 TVAAFHQ LVEDGKIRYWGVSNF DHRALAELQDVPGTSGLTTDQVLYNLSR RGPBY DLLPW 180  
|||| :|:| ||||| ||||| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|  
Db 123 TVAAMEKLIAQGKIRRGWGVSNLDYADMQELWQLPGGNQC ATNQVLYHLGSRGIEY DLLPW 182

Qy 181 CADHQLPV MAYSPIEQ-GR---ILDDTTLNDVAARHSVSPAAA ALAWV LRRDSLCTIPK 235  
| | :| ||||| :| || :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|  
Db 183 CQQQQMPVMAYSPLAQAGRLRNGLLKNAVNEIAHAHNISAAQVLLAWVISHQGVMAIPK 242

Qy 236 ASSPQHVRDNATALDVELTREDLDAL DRAFPSSGPRPLEML 277  
| ::| | :| | | :| :| | :| :| | | | | :| :|  
Db 243 AATIAHVQQNAAVLEVELSSAELAMLDKAYPAPKGKTALDMV 284